Potentials and Pitfalls in Applying ProteinChip SELDI technology to Biomarker Discovery

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Characteristics of Clinical Proteomics Studies

- Person-to-Person variation
- Time-to-Time variation
- Similar causes? HBV? HCV? Alcohol? Aflatoxin?
- Similar progress profile? Accumulation of different mutations and genetic/post-translation modification? Well differentiation? Poor differentiation? Clear cell type?
- Similar symptoms in a disease type?
- Different diseases, but similar symptoms?
Characteristics of Clinical Proteomics Studies

- Large sample sizes
- High-throughput assay
- Quantitative & Qualitative
- Data with complex multi-dimensional relationship
- One disease → one marker? Pattern of markers
SELDI ProteinChip Technology

Retentate Chromatography + MALDI-TOF MS
ProteinChip Reader SELDI-TOF MS (A linear MALDI-TOF MS)
Principle of SELDI ProteinChip System

The SELDI Process and ProteinChip™ Arrays

- Sample goes directly onto the ProteinChip™ Array
- Proteins are captured, retained and purified directly on the chip (affinity capture)
- Retentate Map™ is "read" by Surface-Enhanced Laser Desorption/Ionization (SELDI)
- Retained proteins can be processed directly on the chip

- Proteomic feature defined by mass/charge (m/z) and physico-chemical properties
- Quantitative
- Normalized peak intensity \( \propto \) concentration
- CV: 15% to 30%
- High throughput
High-throughput in 96-well format
Analysis of Mass Spectra –
Peak Matching & Quantification
High-Throughput Quantitative Proteomic Profiling by SELDI-TOF MS (ProteinChip System)

Plasma from diseased and control groups are added onto the ProteinChip Arrays.

After washing, the proteins retained on the chip surface are analyzed with the ProteinChip reader.

Identification of disease-specific proteomic features by comparing the proteomic profiles with bioinformatic tools.

ProteinChip Reader (SELDI-TOF MS)

Disease tissue samples

Normal tissue Samples (control)

Different chip surfaces retaining different types of proteins → Enhance sensitivity

Bioinformatic Analyses

Disease-Specific Proteomic Features
## SELDI vs 2D PAGE (complementary)

<table>
<thead>
<tr>
<th></th>
<th>SELDI</th>
<th>2D PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Throughput</strong></td>
<td>High (PBS II: 176 assays per day)</td>
<td>Low (12 gels per 3 days)</td>
</tr>
<tr>
<td><strong>CV of Normalized Intensity</strong></td>
<td>15 – 30%</td>
<td>30 – 40%</td>
</tr>
<tr>
<td><strong>Best resolving range</strong></td>
<td>2 - 20 kDa</td>
<td>10 – 250 kDa</td>
</tr>
<tr>
<td><strong>Resolving glycoforms/isoforms</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Cost for comprehensive profiling (duplicate)</strong></td>
<td>US$600 [100], ~2500 features [1200] (US$12.5 x 6 x 4 x 2) [12.5 x 4 x 2]</td>
<td>US$75, ~ 2000 features (US$37.5 x 2)</td>
</tr>
<tr>
<td><strong>Recovering differential features for Protein ID work</strong></td>
<td>Non-recovery</td>
<td>Easy to recover (gel spots)</td>
</tr>
<tr>
<td><strong>Time for Protein ID work</strong></td>
<td>1 month to 3 moths Protein purification + 2D-PAGE $\rightarrow$ gel spot $\rightarrow$ trypsin digestion $\rightarrow$ MS</td>
<td>2 days trypsin digestion $\rightarrow$ MS</td>
</tr>
</tbody>
</table>
Use of Proteomic pattern in Serum to Identify Ovarian Cancers

- Proteomic features defined by mass/charge & physico-chemical properties (SELDI-TOF MS)
- Identification of Ovarian Cancer
- The algorithm identified a cluster pattern that, in the training set, completely segregated cancer from noncancer.
- key M/Z values 534, 989, 2111, 2251, and 2465.
- Sensitivity of 100% (95% CI: 93 – 100)
- Specificity of 95% (87 – 99)
Application of SELDI ProteinChip Technology to Discovery of Biomarkers for Various Diseases

Liver Fibrosis and Liver Cirrhosis

Liver Cancer

Gastric Cancer

Nasopharyngeal Carcinoma

Severe Acute Respiratory Syndrome (SARS)
Poon TCW, Chan KCA, Ng PC, Chiu RWK, Ang IL, Tong YK, Ng EKO, Cheng FWT, Li AM, Hon EKL, Fok TF, Lo YMD. Serial Analysis of Plasma Proteomic Signatures in Pediatric Patients with Severe Acute Respiratory Syndrome and Correlation with Viral Load. Clinical Chemistry 50: 1452-1455. 2004.


To GOOD to be TRUE?
Use of Proteomic pattern in Serum to Identify Ovarian Cancers

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- Identification of Ovarian Cancer

- The algorithm identified a cluster pattern that, in the training set, completely segregated cancer from noncancer.

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- Specificity of 95% (87 – 99)
Proteomic diagnostics tested

Concerns about a cancer diagnostics test based on proteomics highlight the threat to public trust in healthcare products where the relevant data are not publicly available — and what can be achieved when they are.

It is easy for critics to slam other scientists who go out on a limb and make big claims for their work. This is the situation facing Emanuel Petricoin and Lance Liotta, two researchers with the US government. The pair led research to develop a ground-breaking diagnostic test for ovarian cancer. Their critics allege that the test is flawed and that corporate entities are trying to rush it to market anyway. The jury is still out on whether the test will prove to be useful in the clinic. But perhaps the most important aspect of the debate is that it would never have arisen if Liotta and Petricoin had not posted their data on the Internet. The episode underscores the crucial importance of readily available public data for scientific progress and, ultimately, for public health.

Liotta and Petricoin believe that their test diagnoses ovarian cancer before the disease progresses to an incurable stage. Their test uses proteomics and involves examining all the proteins in a drop of blood, scanning for a pattern that marks out cancer patients. In a widely hailed study (E. F. Petricoin et al. Lancet 359, 572–577; 2002), they claimed that their proteomic analysis was highly effective. They posted the data on which they based their conclusions on a government website, followed by two more data sets, allowing others to re-analyse their work. Other researchers have done just that, and claim to find technical problems so troubling that they question the conclusions of the original Lancet paper — and even the validity of any diagnostic test based on proteomics (see page 496).

Petricoin and Liotta have defended the utility of proteomics as a diagnostic tool. They say that a large clinical trial should be conducted before it is marketed to consumers. But the controversy - as it appears on the Lancet paper, Correlori is the major player in the United States, Correlori says that it has licensed Quest Diagnostics and the Laboratory to validate the test and ensure that it works in the country and in many different testing conditions. The test has not released the data on which they base their conclusions.

If OvaCheck works, it could save lives. But if the test fails, it will cause the death of an imperfect diagnostic test are those women who receive false positives will undergo needless testing and treatment, whereas those who receive false negatives will not be able to save their lives. Before OvaCheck is available for general use, its licensees need to publish evidence that proves that the test works on samples from doctors around the country and that other scientists have the opportunity to examine the test samples so that they can determine whether the test gives accurate results. If it really works, the researchers have every confidence that independent evaluators would determine to speed OvaCheck's approval.
COMMENTARY

Analysis of Serum Proteomic Patterns for Early Cancer Diagnosis: Drawing Attention to Potential Problems

Eleftherios P. Diamandis

In a recent update (1) of already impressive data (2), it was reported that the use of proteomic patterns in serum to diagnose ovarian and prostate cancers can achieve perfect diagnostic sensitivity and specificity. A diagnostic sensitivity and specificity of 100% is unprecedented for any tumor marker known to date and, if reproducible, this finding could have a major impact on the way we diagnose cancer in the future. Over the last 2 years, results reported by several groups (2–6) have suggested that such proteomic patterns, particularly those generated by surface-enhanced laser desorption/ionization time-of-flight (SELDI-TOF) mass spectrometry, may facilitate the early diagnosis of various cancers, including those of the ovary, prostate, breast, and bladder. SELDI-TOF proteomic profiling technology has been reviewed (7,8).

The impressive results reported with this new technology were welcomed by scientists, the popular press, the public, and even by politicians (9). Although there has been little published criticism of this methodology (10–12), serious skepticism about its utility has been expressed publicly at various scientific meetings. Many investigators and clinicians have adopted a wait-and-see approach pending the outcomes of prospective clinical studies using this technology which are starting now but will require years to complete.

After reviewing the serum level of various malignancies and the current literature, I have concluded that this is currently used for serum detecting any serum component at ng/mL (12). This range of concentration is 1000-fold higher than the concentrations in the circulation (12). This indicates the discriminatory peaks likely represent high-abundance proteins that are released into the circulation, tumors or their microenvironments.
Reproducibility of SELDI-TOF protein patterns in serum: comparing datasets from different experiments

Keith A. Baggerly*, Jeffrey S. Morris and Kevin R. Coombes

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Advance Access publication January 29, 2004

Noise could differentiate Normals from Cancers?

Analytical and preanalytical biases in serum proteomic pattern analysis for breast cancer diagnosis. 


Our findings demonstrate that specimen collection and processing introduce significant biases in the spectral pattern, such that machine learning algorithms can differentiate between sample source, day that the chips were set up, and days that they were read. In contrast, accuracy of predicting cancer was much poorer.

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**Table 1. Accuracy of 2 machine learning algorithms in diagnosis and prediction of variables associated with the proteomic analysis of serum from patients with breast cancer.**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Majority predictor, %</th>
<th>Accuracy, %</th>
<th>P</th>
<th>Accuracy, %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer (A)</td>
<td>72.8 (3.5)</td>
<td>70.9 (12.0)</td>
<td>0.67</td>
<td>66.7 (11.1)</td>
<td>0.12</td>
</tr>
<tr>
<td>Cancer (A, P)</td>
<td>72.8 (3.5)</td>
<td>72.8 (3.5)</td>
<td>1.00</td>
<td>70.8 (7.7)</td>
<td>0.46</td>
</tr>
<tr>
<td>Cancer (S)</td>
<td>70.0 (4.3)</td>
<td>78.4 (13.4)</td>
<td>0.08</td>
<td>78.1 (15.3)</td>
<td>0.13</td>
</tr>
<tr>
<td>Cancer (S, B)</td>
<td>56.7 (8.2)</td>
<td>67.0 (21.8)</td>
<td>0.19</td>
<td>62.9 (21.6)</td>
<td>0.42</td>
</tr>
<tr>
<td>Day chips read</td>
<td>67.2 (2.3)</td>
<td>97.0 (5.0)</td>
<td>$10^{-28}$</td>
<td>88.3 (9.7)</td>
<td>$10^{-8}$</td>
</tr>
<tr>
<td>Day chips set up</td>
<td>35.9 (3.1)</td>
<td>92.5 (7.1)</td>
<td>$10^{-40}$</td>
<td>80.6 (12.2)</td>
<td>$10^{-17}$</td>
</tr>
<tr>
<td>Source of serum</td>
<td>47.5 (1.8)</td>
<td>84.1 (9.2)</td>
<td>$10^{-18}$</td>
<td>85.3 (9.2)</td>
<td>$10^{-20}$</td>
</tr>
</tbody>
</table>

* Mean (SD).

$^b$ A, all patients in the study (n = 132); P, peaks from literature used for classification (see text); S, subset of patients with reproducible spectra (n = 70); B, patients from clinic B only (n = 36).
Reproducibility issue

Technical

Biological
Reproducibility issue 1 – Technical aspect

- Within assay & Between assays
- Within batch & Between batches (ProteinChip array)
- Within person & Between people
- Within robot & Between robots
- Within ProteinChip reader & Between readers (or same reader but get older)
- Within center & Between centers
Reproducibility issue 2 – Biological aspect

- Are all the diseased and control blood samples processed according to the same SOP?
- Problem of multiple comparisons
- Statistically Significant vs Biologically Significant
- Are the normal healthy subjects good controls?
Reproducibility issue

O. John Semmes,1 Ziding Feng,2 Bao-Ling Adam,1 Lionel L. Banez,3 William L. Bigbee,3 David Campos,8 Lisa H. Cazares,1 Daniel W. Chan,6 William E. Grizzle,7 Elzbieta Izbicka,8 Jacob Kagan,6 Gunjan Malhi,4 Dale McLerran,7 Judd W. Moul,5 Alan Partin,4 Preekala Prasadana,7 Jason Rosenzweig,6 Lori S. Somoll,1 Shie Shyaiyava,3 Sudhir Shyaiyava,3 Ian Thompson,6 Manda J. Walsh,3 Nicole White,4 Marcy Winget,2 Yutaka Yasui,2 Zhen Zhang,6 and Liu Zhu7

Results: When the described standard operating procedures were established at all laboratory sites, the across-laboratory measurements revealed a CV for mass accuracy of 0.1%, signal-to-noise ratio of ~40%, and normalized intensity of 15–36% for the three pooled serum peaks. This was comparable to the intralaboratory measurements of the same peaks. The instrument systems were then challenged with sera from a selected group of 14 cases and 14 controls. The classification agreement between each site and the established decision algorithm were examined by use of both raw peak intensity boosting and ranked peak intensity boosting. All six sites achieved perfect blinded classification for all samples when boosted alignment of raw intensities was used. Four of six sites achieved perfect blinded classification with ranked intensities, with one site passing the criteria of 26 of 28 correct and one site failing with 19 of 28 correct.
Recommendations 1 (technical) for Improving the Reproducibility

ProteinChip Reader
- Adjusting the laser power regularly (weekly in my lab)
  - (similar to most of the laser-based MS instrument, keep deteriorating)
- Use a quality control sample to check and fine tune the MS performance using a gold chip

ProteinChip Array
- Standard operating procedures
- Prefer using a robot if possible
- Use a quality control sample to check the reagents and protocol
- Don’t change protocol in the middle of a study
- Randomize the disease and control samples during assay
- Try to finish the study with min. no. of experiments & using the min. no. of batches of chips. (difficult when the sample size is very large)
Reproducibility issue
Are all the Blood Specimens the SAME?

Blood Processing

- Preferably all (diseased and control) processed in the same lab according to a SOP
- Beware of factors affecting serum/plasma content:
  - Blood collection tube (protease inhibitor, EDTA, heparin, citrate, clotting gel, etc)
  - Elapsed time and temperature between venipuncture and separation of serum/plasma (room temp, 4 °C) (e.g. platelets releasing chemokines at 4 °C)
  - Temperature while processing (room temp, 4 °C)
  - Centrifugal force
  - Degree of hemolysis
- Can be partly solved by a multi-center design
Problems of Data Mining

- 1 assay condition $\rightarrow$ ~ 400 proteomic features (peaks or shoulders)
- 5 to 10 conditions $\rightarrow$ 2000 to 4000 proteomic features
- Comparison of disease and control groups ($n = 10$ to 500)
  - T-test or Mann-Whitney rank sum test
  - at $P = 0.05$ $\rightarrow$ 50 false significant features out of 1000
  - Found 100 differential features $\rightarrow$ 50% false discoveries
  - $\rightarrow$ multiple testing problems

Solution: Using SAM to identify diff. pks at an acceptable False Discovery Rate or q-value
  - FDR < 5% or q-value < 0.05 (even lower cutoff)
  - $\rightarrow$ Confident that what you have found are likely true
Problems of Experimental Design or Systemic Bias

- Identification of features-associated with other characteristics of patient groups, but not associated with disease
  - Control samples collected before breakfast (e.g. volunteer)
  - Disease samples collected after lunch (e.g. cancer patients)
  - Features associated with having lunch! Such as insulin, cholecystokinin
High-Throughput Quantitative Proteomic Profiling by SELDI-TOF MS (ProteinChip System)

Plasma from diseased and control groups are added onto the ProteinChip Arrays.

After washing, the proteins retained on the chip surface are analyzed with the ProteinChip reader.

Identification of disease-specific proteomic features by comparing the proteomic profiles with bioinformatic tools.

Normal tissue samples (control)  
Disease tissue samples

ProteinChip Reader (SELDI-TOF MS)

Disease-Specific Proteomic Features

Pitfall in Single center Case-Control Design!
Tumor Resection

Increase in diagnostic markers

Disappearance of diagnostic markers
Identification of Proteomic Markers for Diagnosis of Gastric Cancer
(Cancer and Control cases from the SAME clinic)

**Discovery Phase**
*(Identification of Potential Diagnostic Markers)*

- Sera from 38 gastric cancer patients
- Sera from 29 normal controls

**Levels reversed in post-operative samples?**
- Yes
  - Potential Markers
  - Diagnostic Models

- No
  - Reject

**Diagnostic Model Construction**

**Validation Phase** *(Independent cases)*

- 40 unseen gastric cancer patients
- 20 unseen normal controls

Discovery of 6 SELDI Peaks associated with Gastric Cancer

Copper Chips
- 446 peaks
- Mass range 900 Da – 250,000 Da

WCX Chips
- 270 peaks

A two-stage design to eliminate proteomic features associated with cancer patient group, but not with the disease.

Stage 1
- 38 gastric cancers
  - Vs 29 controls from same clinic
  - SAM analysis false discovery rate = 0

Stage 2
- 38 pre-op
  - Vs 24 post-op (6 – 26 wks)
  - SAM analysis false discovery rate = 0

83% Rejected

26
Diagnostic Model Development

5 markers

Only a Simple Model is needed.

Log2 (Pk 5.1 kDa)
Log2 (Pk 8.6 kDa)
Log2 (Pk 11.5 kDa)
Log2 (Pk 11.8 kDa)
Log2 (Pk 50.1 kDa)

Linear Regression Model

Diagnostic Index

Training cases: 38 Gastric Cancers
29 Normal controls

Diagnostic index: Gastric cancer = 1
Normal control = 0
Diagnosis of Gastric Cancer:

Independent Validation Results
(Samples NOT used for Biomarker Discovery or Diagnostic model construction)

P < 0.001 (Mann Whitney test)

Sensitivity = 83%; Specificity = 95%
TNM stage and Sensitivity (at 95% specificity)

<table>
<thead>
<tr>
<th></th>
<th>Early stage TNM I/II (n = 10)</th>
<th>Advanced stage TNM III/IV (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>50%</td>
<td>92%</td>
</tr>
</tbody>
</table>
Reversed Levels in 14 Independent Postoperative Samples

$P < 0.001$, Mann Whitney test
Protein Chip Array Profiling Analysis in Patients with Severe Acute Respiratory Syndrome Identified Serum Amyloid A Protein as a Biomarker Potentially Useful in Monitoring the Extent of Pneumonia

Timothy T.C. Yip,1 Joanna W.M. Chan,2 William C.S. Chu,1 Tai-Tong Yip,3 Zheng Wang,5 Ting-Lok Kwan,4 Stephen C.K. Lau,3 Dominic N.C. Tsang,5 John K.C. Chan,7 King-Chung Lee,8 Wai-Wai Cheng,1 Victor W.S. Ma,1 Christine Yip,3 Cadmon K.P. Lim,1 Roger K.C. Ngan,3 Joseph S.K. Au,1 Angel Chan,7 Wilena W.L. Lim,6 and Queen Elizabeth Hospital/Hong Kong Government Virus Unit/Ciphergen SARS Proteomics Study Group2

Proteomic Fingerprints for Potential Application to Early Diagnosis of Severe Acute Respiratory Syndrome

Xinhong Kang,1 Yang Xu,2 Xiaoyi Wei,2 Yong Liang,2 Chen Wang,5 Junhua Guo,2 Yafei Wang,1 Maohua Chen,1 Da Wu,3 Yuchen Wang,2 Shengli Bu,2 Yan Qin,2 Peng Lu,10 Jing Cheng,1 Bai Xiao,6 Liangping Hu,8 Xing Gao,12 Jinghong Liu,6 Yeping Wang,7 Yingzhao Song,1 Liguang Zhang,2 Fengshuang Suo,7 Tongyan Chen,1 Zeyu Huang,1 Yunzhuan Zhao,7 Hong Lu,6 Chunqin Fan,3 and Hong Tang10

Table 3. Biomarker statistics for SARS vs non-SARS spectra and decision tree classification.a

<table>
<thead>
<tr>
<th>m/z</th>
<th>P</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Fold</th>
<th>Protein analysis</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(10e^-5)</td>
<td>(10e^-5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3939.08</td>
<td>0</td>
<td>11.80255</td>
<td>10.26216</td>
<td>0.71233</td>
<td>1.72247</td>
<td>16.57</td>
<td>Training</td>
<td>100.00</td>
<td>97.3</td>
</tr>
<tr>
<td>1471.16</td>
<td>0</td>
<td>2.55070</td>
<td>0.54952</td>
<td>2.55070</td>
<td>0.54952</td>
<td>1.00</td>
<td>Test</td>
<td>97.76</td>
<td>99.01</td>
</tr>
<tr>
<td>1154.28</td>
<td>0</td>
<td>2.18112</td>
<td>2.89383</td>
<td>0.26264</td>
<td>0.68112</td>
<td>8.33</td>
<td></td>
<td>(36/37)</td>
<td>(98/903)</td>
</tr>
</tbody>
</table>

a The 95% confidence intervals were estimated using the principle of binomial distribution. P for sensitivity: the 95% confidence interval was 90.6-100.0% for the training set and 85.8-99.9% for the test set; for specificity, the 95% confidence interval was 90.6-99.7% for the training set and 91.9-96.9% for the test set.
Serum Proteins Associated with Severe Acute Respiratory Syndrome (SARS) in Adult Patients

39 SARS cases

39 Control cases

Compared by SAM analysis
(false discovery rate = 0)

821 features

52 Significantly Increased

55 Significantly Decreased

81% rejected

Correlate with > 2 clinical features/biochemical parameters or correlate with SARS viral load

20 SARS-associated Proteomic Features

Serum Amyloid A Is Not Useful in the Diagnosis of Severe Acute Respiratory Syndrome

To the Editor:
In our present study, we aimed to investigate the concentration (SAA), enhancement (SEI) or by EL

Table 1. Summary of the SELDI peaks corresponding to SAA and serum SAA concentrations in adult SARS patients and adult non-SARS patients who were suspected cases during the SARS outbreak period.

| Mean (minimum–maximum) m/z of the SELDI peak | Theoretical average mass | Protein identity* | Non-SARS | SARS | P* | Correlation (r)² with serum concentration of:
<table>
<thead>
<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>11 439 (11 431–11 448)</td>
<td>11 439</td>
<td>SAA-1 (-RS)</td>
<td>0.25 (0.21)</td>
<td>0.10 (0.10)</td>
<td>0.004</td>
<td>0.912 (&lt;0.0005)</td>
</tr>
<tr>
<td>11 526 (11 514–11 541)</td>
<td>11 526</td>
<td>SAA-1 (-R)</td>
<td>0.78 (0.62)</td>
<td>0.36 (0.43)</td>
<td>0.003</td>
<td>0.923 (&lt;0.0005)</td>
</tr>
<tr>
<td>11 681 (11 657–11 689)</td>
<td>11 862</td>
<td>SAA-1</td>
<td>1.38 (1.19)</td>
<td>0.50 (0.58)</td>
<td>0.001</td>
<td>0.930 (&lt;0.0005)</td>
</tr>
</tbody>
</table>

B. Serum SAA concentrations

Mean (SD) serum SAA concentration measured by ELISA, mg/L

<table>
<thead>
<tr>
<th>Non-SARS (n = 37)</th>
<th>SARS (n = 29)</th>
<th>P*</th>
<th>Correlation (r)² with serum CRP concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>853 (769)</td>
<td>395 (428)</td>
<td>0.003</td>
<td>0.499 (0.011)</td>
</tr>
</tbody>
</table>


Sample size for both non-SARS and SARS patients, n = 39.

Mann-Whitney test.

Spearman rank correlation test (P in parentheses).

CRP, C-reactive protein.
Unique proteomic patterns reflecting complex body response to SARS

Sensitivity = 95%
Specificity = 100%
Subgroups of SARS patients with Different Prognosis

- ICU: $P = 0.013$
- Use of Oxygen: $P = 0.011$
Any really useful biomarkers found by proteomic technologies, and obtained FDA approval?

YES!
Three Biomarkers Identified from Serum Proteomic Analysis for the Detection of Early Stage Ovarian Cancer

Zhen Zhang, Robert C. Bast, Jr., Yinhua Yu, Jinong Li, Lori J. Sokoll, Alex J. Rai, Jason M. Rosenzweig, Bonnie Cameron, Young Y. Wang, Niao-Ying Meng, Andrew Berchuck, Carolien van Haften-Day, Neville F. Hacker, Henk W. A. de Bruijn, Ate G. J. van der Zee, Ian J. Jacobs, Eric T. Fung, and Daniel W. Chan

ABSTRACT

Early detection remains the most promising approach to improve long-term survival of patients with ovarian cancer. In a five-center case-control study, serum proteomic expressions were analyzed on 153 patients with invasive epithelial ovarian cancer, 42 with other ovarian cancers, 166 with benign pelvic masses, and 142 healthy women. Data from patients with early-stage ovarian cancer and healthy women at two centers were analyzed independently and the results cross-validated to discover potential biomarkers. The results were validated using the samples from two of the remaining centers. After protein identification, biomarkers for which an immunoassay was available were tested on samples from the fifth center, which included 41 healthy women, 41 patients with ovarian cancer, and 28 each with breast, colon, and prostate cancers. Three biomarkers were identified as follows: (a) apolipoprotein A1 (down-regulated in cancer); (b) a truncated form of transferrin (down-regulated); and (c) a cleavage fragment of inter-alpha-trypsin inhibitor heavy chain H4 (up-regulated). In independent validation to detect early stage invasive epithelial ovarian cancer from healthy controls, the sensitivity of a multivariate model combining the three biomarkers and CA125 [74% (95% CI, 52–90%)] was higher than that of CA125 alone [65% (95% CI, 43–84%)] at a matched specificity of 97% (95% CI, 89–100%). When compared at a fixed specificity of 83% (95% CI, 61–95%), the specificity of the model [94% (95% CI, 85–98%)] was significantly better than that of CA125 alone [52% (95% CI, 29–65%)]. These biomarkers demonstrated the potential to improve the detection of early stage ovarian cancer used individually for screening (2). Longitudinal studies are under way in Europe, Japan, and the United States to evaluate screening strategies using CA125 and/or transvaginal sonography (3–5) and their impact on overall cancer mortality (6). Preliminary results have shown encouraging evidence of a survival benefit among patients diagnosed through a screening regimen (3).

Reports from retrospective studies have shown that multivariate predictive models combining existing tumor markers improve cancer detection (7, 8). Recent advances in genomic and proteomic profiling technology have made it possible to apply computational methods to detect changes in protein expressions and their association to disease conditions, thereby hastening the identification of novel markers that may contribute to multimarker combinations with better diagnostic performance (9–13).

In this study, we hypothesized that comparison of protein expressions of serum specimens from patients with early-stage ovarian cancer with those from healthy women could lead to the discovery of candidate biomarkers for the detection of early stage ovarian cancer. To ensure that the discovered biomarkers are truly associated with ovarian cancer rather than the result of biases in samples, profiling data of specimens from multiple institutions were used for cross-comparison and independent validation. We additionally determined the protein identities of the discovered biomarkers to allow for additional validation with independent methods and as a first step toward
Multicenter Design

Fig. 1. Diagram of study design and patient flow showing the usage of samples from five academic medical centers. Numbers of samples in individual diagnostic groups from each site are indicated in parentheses. A, biomarker discovery and validation. B, derivation and validation of multivariate nonlinear predictive models.

Zhang et al, Cancer Res 64, 5882-90, 2004
Obtained FDA approval in 2009

U.S. Food and Drug Administration

For Immediate Release: Sept. 11, 2000

Media Inquiries: Peper Long, 301-796-4671, mary.long@fda.hhs.gov
Consumer Inquiries: 888-INFO-FDA

FDA clears a test for ovarian cancer
Test can help identify potential malignancies, guide surgical decisions

The U.S. Food and Drug Administration today cleared a test that can help detect ovarian cancer in a pelvic mass that is already known to require surgery. The test, called OVA1, helps patients and health care professionals decide what type of surgery should be done and by whom.

OVA1 identifies some women who will benefit from referral to a gynecological oncologist for their surgery, despite negative results from other clinical and radiographic tests for ovarian cancer. If other test results suggest cancer, referral to an oncologist is appropriate even with a negative OVA1 result.

OVA1 should be used by primary care physicians or gynecologists as an adjunctive test to complement, not replace, other diagnostic and clinical procedures.

OVA1 uses a blood sample to test for levels of five proteins that change due to ovarian cancer. The test combines the five separate results into a single numerical score between 0 and 10 to indicate the likelihood that the pelvic mass is benign or malignant.

OVA1 is intended only for women, 18 years and older, who are already selected for surgery because of their pelvic mass. It is not intended for ovarian cancer screening or for a definitive diagnosis of ovarian cancer. Interpreting the test result requires knowledge of whether the woman is pre- or post-menopausal.

The American College of Obstetricians and Gynecologists and the Society of Gynecologic Oncologists published recommendations in 2002 for the role of generalist obstetrician-gynecologists in the early detection of ovarian cancer, which included a recommendation of patient referral to a gynecological oncologist when specific indicators of malignancy are present.

These recommendations and later reports indicate that patients with ovarian cancer have improved survival when the surgery is performed by gynecologic oncologists as opposed to general gynecologists or surgeons.

"Tests such as OVA1 personalize and improve public health by providing patients and health care providers with more information to support medical decisions that impact survival rates and reduce surgical complications," said Jeffrey Shuren, M.D., J.D., acting director of the FDA's Center for Devices and Radiological Health.

The FDA reviewed a study of 516 patients, including 260 evaluated by non-gynecological oncologists, which compared OVA1 results with biopsy results. When combined with pre-surgical information, such as radiography and other laboratory tests, results from the OVA1 tests identified additional patients who might benefit from oncology referral who were not identified using pre-surgical information alone.

OVA1 is developed by Vermillion Inc., headquartered in Fremont, Calif., in conjunction with researchers at The Johns Hopkins University in Baltimore.
Common Concerns & Questions from Researchers

- Seems that most of the published data are not reproducible?
  - Reproducible with SOPs when using the same lot of chips

- Limited dynamic range? Without prefractionation, only down to proteins at ug/mL levels?
  - Same for other currently available high-throughput technologies, BUT solutions are coming

- Why most of the identified potential serum biomarkers are acute phase reactants or proteins associated immune response?
  - Same for other currently available high-throughput technologies
What are Diagnostic/Prognostic SELDI proteomic features?

- Produced by tumor (1\textsuperscript{st} events)

- Body \textit{biological} response to tumor (2\textsuperscript{nd} events)

- Body \textit{molecular} response to tumor (2\textsuperscript{nd} events, e.g. secretary tumor glycosidase $\rightarrow \Delta$glycosylation)

- Body changes in response to Body response to tumor (Tertiary events)
Single marker

vs

Multiple markers
(Diagnostic/Prognostic Proteomic Patterns)
Why Proteomic Patterns?

Early diagnosis
- Variation in carcinogenesis pathway
- Small tumor
- Small detectable signals
- 2\textsuperscript{nd} and/or 3\textsuperscript{rd} events
- Signal amplification
- Higher detection sensitivity

Prognosis
- Tumor, not only parameter affecting treatment response and survival
- Body status, e.g. liver function
- Drug adverse effect prediction, e.g. metabolic enzymes in liver
High-throughput proteomic profiling assay
- a future universal blood test for various human diseases

Profiling of serum proteins by using ProteinChip arrays/other technologies

Database of disease proteomic fingerprints

Serum protein fingerprints

Analyzed by computer
Tips for a Successful SELDI Study

- Standard operation procedure (fixed incubation time, fixed washing time, fixed dilution fold, etc.)

- Both disease and control samples collected at a well-controlled environment (same clinic)

- Infectious diseases:
  - Control cases with different infectious diseases, but similar symptoms
  - Similar timing of sample collection

- Conservative data mining: stringent criteria for a proteomic features regards as disease-specific
  - Correlation with the pathogen load
  - Reverse upon the recovery (longitudinal follow-up design)

- Validate by cases from other hospitals (i.e. Multi-centre study)
Concept of Serum Proteomic Fingerprinting
→ Not restricted to MS-based technologies


Serum Protein Electrophoresis – Earliest Type of Serum Protein Electrophoresis

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Characteristic Patterns of Acute-Reaction Proteins Found on Serum Protein Electrophoresis and Associated Conditions or Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased albumin</td>
<td>Increased beta_1 or beta_2 globulins</td>
</tr>
<tr>
<td>Dehydration</td>
<td>Biliary cirrhosis</td>
</tr>
<tr>
<td>Decreased albumin</td>
<td>Carcinoma (sometimes)</td>
</tr>
<tr>
<td>Chronic cachectic or wasting diseases</td>
<td>Cushing’s disease</td>
</tr>
<tr>
<td>Chronic infections</td>
<td>Diabetes mellitus (some cases)</td>
</tr>
<tr>
<td>Hemorrhage, burns, or protein-losing enteropathies</td>
<td>Hypothyroidism</td>
</tr>
<tr>
<td>Impaired liver function resulting from decreased synthesis of albumin</td>
<td>Iron deficiency anemia</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>Malignant hypertension</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>Nephrosis</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Polyarteritis nodosa</td>
</tr>
<tr>
<td>Increased alpha_1 globulins</td>
<td>Obstructive jaundice</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Third-trimester pregnancy</td>
</tr>
<tr>
<td>Decreased alpha_1 globulins</td>
<td>Increased beta_1 or beta_2 globulins</td>
</tr>
<tr>
<td>Alpha-antitrypsin deficiency</td>
<td>Protein malnutrition</td>
</tr>
<tr>
<td>Increased alpha_2 globulins</td>
<td>Increased gamma globulins</td>
</tr>
<tr>
<td>Adrenocorticosteroid therapy</td>
<td>Amyloidosis</td>
</tr>
<tr>
<td>Advanced diabetes mellitus</td>
<td>Chronic infections (granulomatous diseases)</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>Chronic lymphocytic leukemia</td>
</tr>
<tr>
<td>Decreased alpha_2 globulins</td>
<td>Cirrhosis</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>Hodgkin’s disease</td>
</tr>
<tr>
<td>Megaloblastic anemia</td>
<td>Malignant lymphoma</td>
</tr>
<tr>
<td>Protein-losing enteropathies</td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>Severe liver disease</td>
<td>Rheumatoid and collagen diseases (connective tissue disorders)</td>
</tr>
<tr>
<td>Wilson’s disease</td>
<td>Waldenström’s macroglobulinemia</td>
</tr>
<tr>
<td></td>
<td>Decreased gamma globulins</td>
</tr>
<tr>
<td></td>
<td>Agammaglobulinemia</td>
</tr>
<tr>
<td></td>
<td>Hypogammaglobulinemia</td>
</tr>
</tbody>
</table>

Automated Paragon 2000 clinical capillary zone electrophoresis (CZE) system

Fig. 1. Examples of measurements performed in the electropherogram.
Take Home Messages

- SELDI is a useful technology for quantitative proteomic profiling.

- Good experimental design and statistical concepts help finding real differential peaks.

- Standard operating procedure
  - □ → technically reproducible
  - □ → increase precision → increase statistical power

- SELDI ProteinChip reader is NOT just for Proteomic Profiling (Stereotyped)
  - □ Quantitative glycomic profiling
    - □ New types of ProteinChip array for glycan profiling (e.g. Sepharose or Graphite ProteinChip array)
  - □ SEND chip for peptidomic profiling
  - □ DNA-protein, protein-protein interaction partners
  - □ Multiple MS-immunoassay
  - □ etc.

- Other new technologies for serum proteomic profiling are coming.
Proteomic Research –
A difficult and expensive journey need supports from colleagues and various parties

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  Dept of Anatomical and Cellular Pathology (HK Ng, Nathalie Wong)
  Dept of Chemical Pathology (Dennis Lo, Allen Chan, Rossa Chiu)
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Thank you!